IN THE FIGURES:

The figures of the application has been amended as follows:

In the title to Figure 20a, "(SEQ ID NO:137)" has been replaced with --(SEQ ID NO:141)--.

In the title to Figure 20b, "(SEQ ID NO:142)" has been replaced with --(SEQ ID NO:146)--.

Remarks

The preceding amendments and the following remarks are provided to clearly point out the appearance and descriptions of the SEQ ID NOs identified in the communication from the Examiner, pursuant to 37 CFR 1.821.

SEQ ID NO: 19- In the amendment put forth in paper number 12, on page 2, at line 17, SEQ ID NO:19 is identified as amino acids 20-95 of mouse neurturin, which is the pre-pro region of mouse neurturin.

SEQ ID NO: 102- In the specification, on page 101, at line 39, SEQ ID NO:102 is described as the reverse primer used to generate the 210 nucleotide DNA fragment from genomic DNA, which was used as a probe in subsequent experiments.

SEQ ID NOS:125-129- These sequences are first introduced in the specification, on page 3, at line 28. The amendment described herein should clarify that these sequences are short peptides, derived from persephin, to which degenerate primers are made.

SEQ ID NO:180- This sequence is cited in the specification-as-filed, on page 56, at line 12. In the amendment presented in this paper, SEQ ID NO:180 is clearly defined as the complementary sequence of a mouse cDNA, which encodes the persephin open reading frame.

SEQ ID NO:191- This sequence is cited in the specification-as-filed, on page 56, at line 12. In the amendment presented in this paper, SEQ ID NO:191 is clearly defined as the complementary sequence of a rat cDNA, which encodes the persephin open reading frame.

SEQ ID NO:206- This sequence is cited in the specification-as-filed, on page 56, at line 13. In the amendment presented in this paper, SEQ ID NO:206 is clearly defined as the complementary sequence of a human cDNA, which encodes the persephin open reading frame.

As per Examiner's recommendation, the specification has been amended to include SEQ ID NOS:141 and 146 in the "Brief Description of Drawings" section of the specification. Furthermore, the heading to Figure 20b has been amended to replace the SEQ ID NO:142 citation with a reference to SEQ ID NO:146. The citation of these sequence identification numbers in the body of the specification

and the change in Figure 20b do not constitute new matter, since SEQ ID NO:141 is described on page 115 at lines 35-36 and SEQ ID NO:146 is described on page 116 at lines 32-33 of the application-as-filed.

Additionally, page 56 of the specification has been amended to clarify what exactly the "reference sequences" (SEQ ID NOS:179, 180, 190, 191 and 203-206) are. As the sequences were already disclosed in the sequence listing of the application-as-filed, the clarification of the references in the body of the text does not constitute new matter.

Furthermore, applicants submit revised copies of pages 10, 11, 35 and 56, which include all of the prior amendments, and marked-up copies of Figures 20A and B.

In view of the amendments and remarks presented above, and the submission of substitute text and marked-up figures, it is believed that applicants have complied with all of the issues presented in papers number 14 and 16, and with the requirements of 37 C.F.R. §§1.821-1.825 and 37 CFR §1.121(a) and (b).

Respectfully submitted,

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Figure 16 illustrates the sequences of TGF-β superfamily members aligned using the Clustal method, from the first canonical framework cysteine to the end of the sequence for transforming growth factor-β1 (TGFβ1)(SEQ ID NO: 150), transforming growth factor-β2 (TGFβ2)(SEQ ID NO: 151), transforming growth factor-β3 (TGFβ3)(SEQ ID NO: 152), inhibin β A (INHβA)(SEQ ID NO: 153), inhibin β B (INHβB)(SEQ ID NO: 154), the *nodal* gene (NODAL)(SEQ ID NO: 155), bone morphogenetic proteins 2 and 4 (BMP2 and BMP4)(SEQ ID NO: 156 AND 157, respectively), the *Drosophila decapentaplegic* gene (dpp)(SEQ ID NO: 158), bone morphogenetic proteins 5-8 (BMP5, BMP6, BMP7 and BMP8)(SEQ ID NO: 159, 160, 161 and 162, respectively), the *Drosophila* 60A gene family (60A)(SEQ ID NO: 163), bone morphogenetic protein 3 (BMP3)(SEQ ID NO: 164), the *Vg1* gene (SEQ ID NO: 165), growth differentiation factors 1 and 3 (GDF1 and GDF3)(SEQ ID NO: 166 and 167, respectively), dorsalin (drsln)(SEQ ID NO: 168), inhibin α (INHα)(SEQ ID NO: 169), the *MIS* gene (MIS)(SEQ ID NO: 170), growth factor 9 (GDF-9)(SEQ ID NO: 171), glial-derived neurotropic growth factor (GDNF)(SEQ ID NO: 172) and neurturin (NTN)(SEQ ID NO: 173);

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Figure 17 illustrates (A) full length murine persephin gene (SEQ ID NO:177 and the reverse complement sequence SEQ ID NO:178) with arrows indicating an 88 nt intron from positions 155-242 and (B) the nucleotide sequence of murine pre-pro persephin (SEQ ID NO:179) with encoded amino acid sequence (SEQ ID NO:185);

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Figure 18 illustrates (A) full length rat persephin gene (SEQ ID NO:188 and the reverse complement sequence SEQ ID NO:189) with arrows indicating an 88 nt intron from positions 155-242 and (B) the nucleotide sequence of rat pre-pro persephin (SEQ ID NO:190) with encoded amino acid sequence (SEQ ID NO:196);

Figure 19 illustrates a western blot analysis using anti-persephin antibodies to detect persephin protein in cell lysates from COS monkey cells transfected with the murine persephin gene (lane 2) or the rat persephin gene (lane 3) compared to cells transfected

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with the non-recombinant vector alone (pCB6, lane 4) and the mature protein produced by *E. Coli* (lane 1);

Figure 20 illustrates the murine chimeric molecules (A) PSP/NTN containing the persephin fragment (residues 1-63) and the neurturin fragment (residues 68-100)(SEQ ID NO:141) and (B) NTN/PSP containing the neurturin fragment

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(residues 1-67) and the persephin fragment (residues 64-96)(SEQ ID NO:146) with the arrow indicating the crossover point in each;

Figure 21 illustrates the survival promoting effect of persephin in murine embryonic day-14 mesencephalic cells cultured for three days (a) in the absence of persephin where almost all of the cells are dead and (b) in the presence of persephin (100 ng/ml) where substantial neuronal cell survival is evident;

Figure 22 illustrates the survival promoting effect of persephin (PSP) in murine embryonic day-14 mesencephalic cells compared to effects of neurturin (NTN) and GDNF, measured by the number of cells stained with tyrosine hydroxylase (TOH);

Figure 23 illustrates RT/PCT survey for persephin expression in adult mouse tissues showing persephin expression by Kidney cells; and

Figure 24 illustrates the cDNA sequence of human pre-pro persephin (SEQ ID NO:203) with two silent mutations indicated at positions 30 and 360 and the encoded amino acid sequence (SEQ ID NO:217) with the first amino acid of the pro- region indicated by the double asterisks (**) at amino acid position 24 and the first amino acid of mature human persephin indicated by the single asterisk (*) at amino acid position 61.

Description of the Preferred Embodiments

The present invention is based upon the identification, isolation and sequencing of a DNA molecule that encodes a new growth factor, persephin. Persephin promotes cell survival and, in particular, the survival of neuronal cells. Prior to this invention, persephin was unknown and had not been identified as a discrete biological substance nor had it been isolated in pure form.

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neurturin and persephin make possible this powerful new approach which can now successfully identify other gene family members. Using this new approach, one may screen for genes related to GDNF, neurturin and persephin in sequence homology by preparing DNA or RNA probes based upon the conserved regions in the GDNF and neurturin molecules. Therefore, one embodiment of the present invention comprises probes and primers that are unique to or derived from a nucleotide sequence encoding such conserved regions and a method for identifying further members of the neurturin-persephin-GDNF gene family.

Conserved-region amino acid sequences have been identified herein to include Val-Xaa_1-Xaa_2-Leu-Gly-Leu Gly-Tyr where Xaa_1 is Ser, Thr or Ala and Xaa_2 is Glu or Asp (SEQ ID NO:108); Glu-Xaa_1-Xaa_2-Xaa_3-Phe-Arg-Tyr-Cys-Xaa_4-Gly-Xaa_5-Cys in which Xaa_1 is Thr, Glu or lys, Xaa_2 is Val, Leu or Ile, Xaa_3 is Leu or Ile, Xaa_4 is Ala or Ser, and Xaa_5 is Ala or Ser, (SEQ ID NO:113); and Cys-Cys-Xaa_1 Pro-Xaa_2-Xaa_3-Xaa_4-Xaa_5-Asp-Xaa_6-Xaa_7-Xaa_8-Phe-Leu-Asp-Xaa_9 in which Xaa_1 is Arg or Gln, Xaa_2 is Thr or Val or Ile, Xaa_3 is Ala or Ser, Xaa_4 is Tyr or Phe, Xaa_5 is Glu, Asp or Ala, Xaa_6 is Glu, Asp or no amino acid, Xaa_7 is val or leu, Xaa_8 is Ser or Thr, and Xaa_9 is Asp or Val (SEQ ID NO:114). Nucleotide sequences containing a coding sequence for the above conserved sequences or fragments of the above conserved sequences can be used as probes. Exemplary probe of primer sequences encoding amino acid sequences of SEQ ID NOS:125-129; primers whose reverse complementary sequences encode amino acid sequences SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:130; and, in particular, nucleotide sequences, SEQ ID NOS:115-124. Additional primers based upon GDNF and neurturin include nucleic acid sequences encoding amino acid sequences, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:40 and SEQ ID NO:41; primers whose reverse complementary sequences encode SEQ

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expressing the persephin gene. Certain tissues such as those identified below in example 18 have been found to express the persephin gene. The method comprises hybridizing a polynucleotide probe to mRNA from a sample of tissue that normally express the persephin gene or from a cDNA produced from the mRNA of the sample. The sample is obtained form a patient suspected of having an abnormality in the persephin gene or from a particular patient tissue or cell type suspected of having an abnormality in the persephin gene. The reference persephin polynucleotide probe can comprise a cDNA encoding the complete mouse persephin open reading frame SEQ ID NO:179, or the complement thereof, SEQ ID NO:180); a cDNA encoding the complete rate persephin open reading frame (SEQ ID NO:190 or the complement thereof, SEQ ID NO:191); cDNA encoding complete human persephin open reading frames (SEQ ID NOS:203 or 205, or their respective complements, SEQ ID NOS:204 or 206) or derivatives thereof or fragments thereof so long as such derivatives or fragments specifically hybridize to persephin mRNA or from a cDNA produced from a persphine mRNA.

G4 Cont

To detect the presence of mRNA encoding persepin protein, a sample is obtained from a patient. The sample can be from blood or from a tissue biopsy sample. The sample may be treated to extract the nucleic acids contained therein. The resulting nucleic acid from the sample is subjected to gel electrophoresis or other size separation techniques.

The mRNA of the sample is contacted with a nucleic acid serving as a probe to form hybrid duplexes. The use of a labeled probes as discussed above allows detection of the resulting duplex.

When using cDNA encoding persephin protein or a derivative of the cDNA as a probe, high stringency conditions can be used in order to prevent false positives, that is the hybridization and apparent detection of persephin nucleotide sequences when in fact an intact and functioning persephin gene is not present.

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When using sequences derived from the persephin cDNA, less stringent conditions could be used, however, this would be a less preferred approach because of the

FIGURES AS AMENDED

FIGURE 20A

PSP/NW (SEQ ID NO: 141)

ALAGSCRLWSLTLPVAELGLGYASEEKVIFRYCAGSCPQEARTQHSLVLA 50

RLRGRGRAHGRPCCRPTAYEDEVSFLDVHSRYHTLQELSARECACV 96

FIGURE 20B

NTH/PSP (SEQ ID NO:146)

PGARPCGLRELEVRVSELGLGYTSDETVLFRYCAGACEAAIRIYDLGLRR 50

LRQRRRVRRERARAHPCCQPTSYADVTFLDDQHHWQQLPQLSAAACGCGG 100